PATHOLOGY AND PATHOGENESIS OF VIRAL HEPATITIS

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INTRODUCTION

The prevalence of viral hepatitis, including hepatitis A and B, is markedly different from country to country. The incidence of hepatitis in Asian and African countries is very high compared to that in European countries and the United States.

Hepatitis A is most predominant and appears to be endemic in tropical countries, such as Southeast Asia, Africa and Mediterranean area, for instance Greece. On the other hand, in Japan, there is no large epidemics for over twenty years, at present only a few small outbreaks in special condition and a few sporadic cases have been reported.

Concerning hepatitis B, mass survey has already been performed all over the world. Positive rates of HBsAg in the general population are markedly different in areas. The highest positive rate, as high as 15%, was reported from Southeast Asia and Africa. In Japan, the rate is 2-3% and 0.1-0.2% in the United States. Therefore,120 million people are estimated carrying hepatitis B virus in the world. Hepatitis B virus causes persistent infection and relates with chronic hepatitis, hepatitic cirrhosis and hepatocellular carcinoma.

Hepatitis A and B virus and the pathogenesis of viral hepatitis is discussed herein.

Hepatitis A

Hepatitis A virus, 27nm in diameter, is detected in faeces which is obtained just before the onset of hepatitis. Therefore it is very difficult technically to get the faeces containing hepatitis A virus. Anti HA antibody can be tested by IAHA method using purified HA antigen. Anti HA antibody in sera of various age groups in Japan was examined. Positive rate of anti HA antibody is very low in cases under twenty years of age, as low as 5%. This phenomenon suggests that there have been no large epidemics for those twenty years. However, positive rate goes up suddenly at age of thirty and continues high level until old age groups, as high as 90%. This also means almost all Japanese people were infected during the Second World War or after the War. On the other hand, in Sri Lanka, positive rate of HA antibody was already high in young children. Therefore, many people in Southeast Asia might be infected with hepatitis A virus in childhood (Fig. 1).

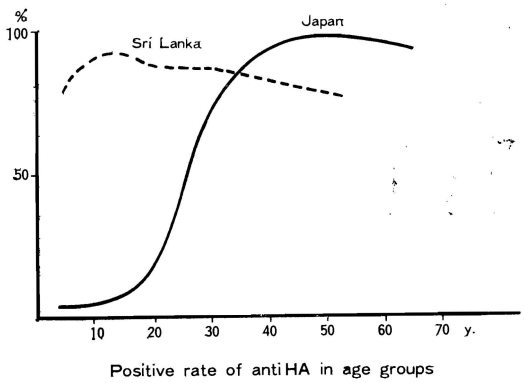

Fig. 1 —Positive rate of anti HA antibody in various age groups in Japan and Sri Lanka.

In Japan, only five or six small outbreaks of hepatitis A was reported for a couple of years which were observed in special conditions, such as in institution for mental retarded persons and some nursery schools.

However, we have still a few sporadic hepatitis A cases and half of them got HA virus

infection during their trip in Southeast Asia. This hepatitis A virus infection might be prevented by administration of conventional γ-globulin. Commercial γ-globulin contains enough anti HA antibody. Histological features of hepatitis A is not different from hepatitis B.

Hepatitis B

Hepatitis B virus is round particle measuring 42 nm in diameter and consists of core and surface substance, which are called as HBcAg and HBsAg respectively. Excess HBsAg is produced by the hepatocytes and excreted into the blood as small particle measuring 20 nm in diameter. Core particles proliferate in the nucleus of the hepatocyte and HBsAg is formed in the cytoplasma. HBcAg can be demonstrable by immunofluorescent method as well as immunoperoxidase method in the nuclei of hepatocytes. Electronmicroscopically it appeares as numerous round particles in the nucleoplasma (Fig. 2). On the other hand, HBsAg is seen in the cytoplasma by means of immunofluorescent method, immunoperoxidase method and orcein stain (Fig. 3). Various distribution patterns of HBsAg are observed, such as diffuse pattern or cytoplasmic inclusion body. Sometimes HBsAg is seen along cell membrane of the hepatocytes. Electronmicroscopical feature of HBsAg is not yet completely clear. Although we can see proliferation of smooth endoplasmic reticulums and filamentous structures in them in the hepatocytes containing HBsAg, (Fig. 4) it is not clear filamentous structures are HBsAg itself or not.

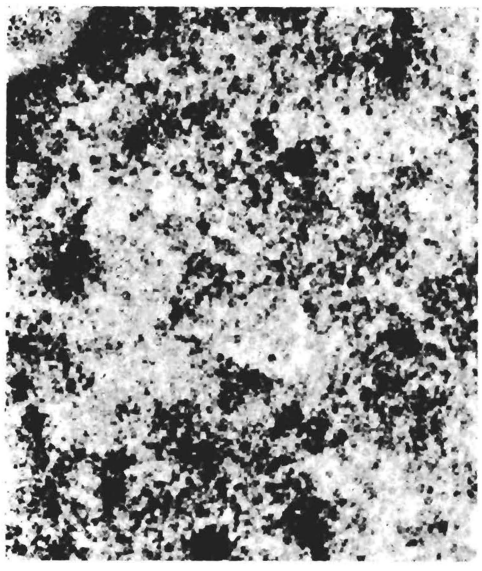

Fig. 2 —Hepatitis B core particles in the nucleus of hepatocyte.

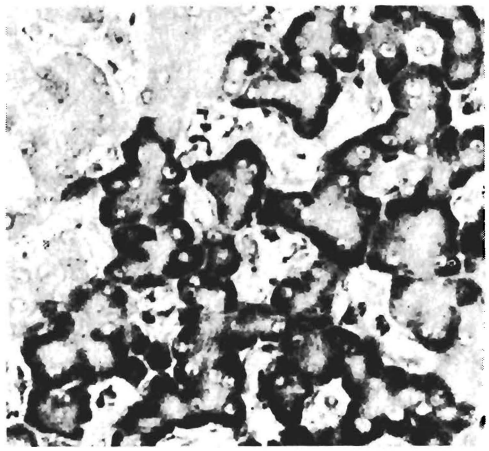

Fig. 3 —HBsAg in an area of the liver lobule. Orcein stain.

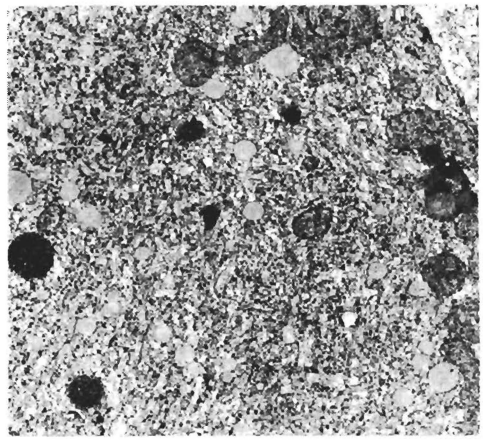

Fig. 4 —Electronmicroscopical feature of a hepatocyte containing HBsAg.

The pathogenesis of liver cell injury in hepatitis B has been the subject of conjectures and hypothesis. It has been presumed that direct cytopathic effects of the hepatitis B virus itself are less likely for the pathogenesis; because of the presence of an asymptomatic chronic HBAg carrier with abundant HBsAg in the cytoplasm and HBcAg in the nucleus of hepatocytes and yet no enzymatic and histological evidences of hepatocyte necrosis.

On the other hand, in acute hepatitis as well as chronic active hepatitis, hepatitis B virus antigenetic materials in the liver tissue are rather scanty. Especially in fulminant hepatitis, demonstrable HBsAg in the liver tissue by immunofluorescent method is very scanty. That is, hepatitis B virus in the liver tissue and the extent of liver cell damage does not correlate and rather shows reverse correlation.

Therefore, it appears now in general agreement that host immune response to hepatitis B virus or its associated antigens is responsible for the pathogenesis of liver-cell necrosis in hepatitis B. The immune humoral response against HBsAg could be incriminated as one of the important factor in the pathogenesis of acute hepatitis. In acute hepatitis, especially fluminant type, HBsAg immune complexes occur in the blood and immune complex is demonstrated in the blood vessels, glomeruli, etc. by immunofluorescent method. Complement levels also decline in this stage.

In fluminant hepatitis, marked liver cell destruction is seen, whereas HBV associated antigens in the liver tissue are very scanty. On the other hand, immunoglobulin especially IgG and complement is abundantly demonstrated in the necrotic areas.

Anti HBs producing cells in the lymphoid and liver tissue are stainable by immunofluorescent antigen method. Numerous anti HBs producing cells were observed in the lymphoid tissue in the cases with fluminant hepatitis, even we found abundant HBsAg in the blood.

Cellular immune reaction also appears to play an important role in the HBV infection and the liver cell injury. Since the author developed new staining methods of HBsAg in paraffin sections, we have expected to find histological evidences suggesting T lymphocyte reaction against HBsAg-containing hepatocytes. However, in the usual cases of acute and chronic hepatitis, such findings were rare. Usually lymphocytes infiltrate around necrotic hepatocytes without stainable HBsAg by the orcein stain. Acidophilic bodies do not always contain HBsAg. However, if we select the case, we can find the histopathological findings suggesting the morphological expression of killer-lymphocytes or immunologically activated T lymphocytes in action against the liver cell with HBsAg in the cytoplasm (Fig. 5). In those cases, small lymphocytes and macrophages in close contact with HBsAg containing hepatocytes were observed. Variable degenerative changes, such as shrinkage, partial lysis, lytic necrosis and complete drop-out, were demonstrated in those HBsAg containing hepatocytes. The earliest change consists of slightly degenerated

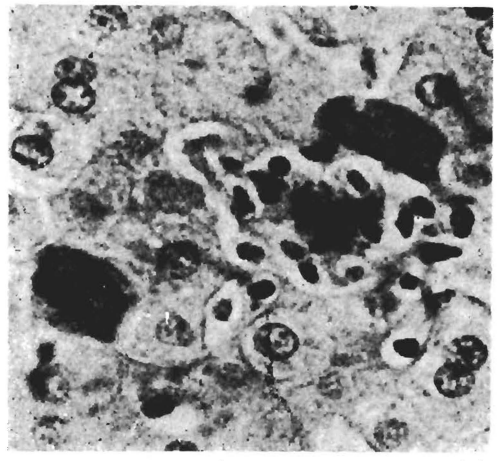

Fig. 5 —Spotty necrosis of an hepatocyte containing HBsAg, surrounded by a few lymphocytes and macrophages.

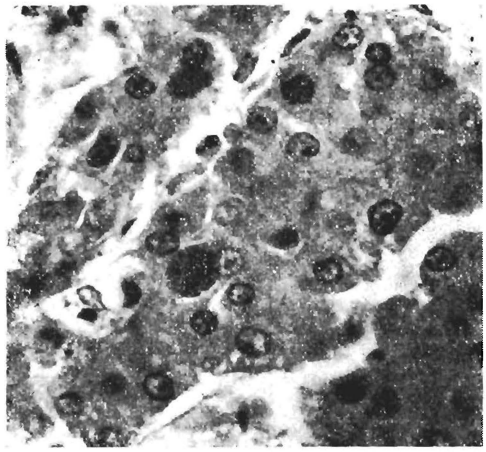

Fig. 6 —HBsAg in the tumor cell of hepatoma. Orcein stain.

liver cell retaining the cell contour with one or two small lymphocytes in contact. The cytoplasm is still finely reticulated by delicate networks of orcein positive material. Slightly advanced one is moderate cytolysis of a hepatocyte with the still traceable cell contour and one or two lymphocytes and macrophages in contact. The cytoplasm is coarsely reticulated with thicker and irregular somewhat clumpy, networks of the orcein positive material. Further advanced one is small granulomatous aggregation of several macrophages and a few lymphocytes with no liver cell contour at all. Irregular-sized and shaped clumpy coagula of the orcein-positive material are phagocytosed by macrophages. Those findings, such as killer lymphocytes in action against HBsAg containing hepatocytes, are also observed under the electron microscope. Some lymphocytes are migrating near the liver cells with the numerous filamentous structures in the proliferated smooth endoplasmic reticulums. Pseudopods of the lymphocytes appear to be directed towards those liver cell through endothelial pores. In areas, a small lymphocyte is in contact to an obviously injured liver cell.

Those findings show that the cellular immune response can injure liver cells branded by HBsAg.

We are now performing animal experiment of type B hepatitis. Chimpanzee is only one susceptible animal for hepatitis B virus except for a human being. We found in this experiment that infectivity of sera containing HB antigen are very different between serum containing e antigen and serum containing e antibody. e antigen positive serum is highly infectious and its 50% chimpanzee infectious dose was about 10^{-8}, whereas e antibody positive serum is less infectious and its infectivity 10^{0}. Therefore infectivity of both sera is different tremendously. This evidence might explain the mode of vertical transmission of hepatitis B virus, from mother to child. If the mother of HB carrier has e antigen, she transmitts hepatitis B virus to her children in high rate. However, if the mother of HB carrier has e antibody, she does not transmit virus to her children.

We found also another evidence, that is, dose of inoculated virus does not related with severity of the disease. It just relates to incubation period. If inocula contains larger amount of virus, incubation period is shorter. Whereas if we give small amount of virus, incubation period is very long.

In this experiment, we can produce typical acute type B hepatitis and chronic hepatitis and asymptomatic hepatitis B carrier in chimpanzees.

Hepatocellular carcinoma (hepatoma) and hepatitis B infection

Hepatoma in human being has an intimate causal relationship with liver cirrhosis, since they are frequently associated with each other. Among various types of liver cirrhosis, hepatoma is usually complicated with macronodular cirrhosis, designated as post-hepatitic or hepatitic cirrhosis, whereas its complication rate with other types of cirrhosis, including

micronodular or alcoholic cirrhosis is rather low, except for hemochromatosis.

For instance, in Japan, about 50%of cases with hepatitic cirrhosis give rise to hepatoma, whereas its rate in alcoholic cirrhosis is less as 12 %. Those phenomena are essentially similar in geographic areas that have a high incidence of hepatoma and those with a low incidence.

Differences of geographical distribution of liver cirrhosis and that of hepatoma, and different complication rates of hepatoma among cirrhotics from country to country could be explained from the difference in the predominant type of cirrhosis. In European countries and the United States, predominant type of cirrhosis is alcoholic and in those countries incidence of hepatoma is very low. On the other hand, in Asia and African countries, predominant cirrhosis is hepatitic and hepatoma is a very common cancer.

Complication of the hepatoma with other types of liver cirrhosis is very rare, except for hemochromatosis.

However, hepatoma might develop not only on the basis of liver cirrhosis but also on the chronic persistent hepatitis. Because, in autopsy materials, about 85% of hepatoma are complicated with liver cirrhosis. Some cases of hepatoma are complicated also with mild liver fibrosis. Those cases are showing very mild portal fibrosis with scanty cell infiltration. We examined HBsAg in those cases by orcein stain. Positive reaction is seen in some cases. HBsAg was found in 40% of cases of liver fibrosis with hepatoma. Thus it may be presumed that some of the liver fibrosis associated with hepatoma was related to hepatitis B and those were explained as chronic persistent hepatitis. Previously, it was thought by most pathologists that hepatoma only occurred in fully developed macronodular cirrhosis and destruction and regeneration of the hepatocytes was incriminated as one of the important role for hepatocarcinogenesis. However, hepatoma may develop not only on the basis of cirrhosis but also chronic persistent hepatitis, in which destruction of the hepatocytes is minimal. This evidence is also suggesting carcinogenicity of hepatitis B virus.

The seropositivity rate for HBsAg of patient with hepatoma is very high compared to the control groups. It is 40-60% in Japan depending on the areas, and as high as 80% has been reported from Taiwan and Uganda. Those findings are highly suggesting carcinogenetic activity of hepatitis B virus.

Serum titer of HBsAg in cases with hepatoma is usually low. However, HBsAg is demonstrable abundantly in non-cancerous liver tissue as demonstrated by immunofluorescent technique or orcein stain. We investigated HBV associate antigens in the liver tissue of hepatoma cases by means of orcein method for HBsAg and peroxidase antibody method for HBcAg. Examinations were carried out in total 47 cases with hepatoma, of which 25 cases were HBAg negative at least in the liver tissue, and in 22 cases, we found HBsAg in the liver tissue. HBcAg was demonstrated only in 7 cases. HBsAg and HBcAg is restricted in non-cancerous hepatocytes in usual hepatoma case, and hepatoma cells do not contain HBsAg. In only few cases, especially well developed hepatoma, HBsAg and HBcAg is also demonstrable in hepatoma cells (Fig. 6). We only found two such cases among 22 HBAg positive hepatoma cases. In one of such cases, we found three histologically different hepatoma tissue, well-differentiated green hepatoma, well-differentiated white hepatoma, and infiltrating rather anaplastic hepatoma. We found HBsAg and HBcAg only in well-differentiated one, and no hepatitis B virus associated antigen expression in anaplastic hepatoma. Our working hypothesis on hepatocarcinogenesis associated with hepatitis B virus is as follows: in

acute and in active stage of chronic hepatitis, HBsAg containing hepatocytes are removed by immunological processes. Persistent, long-term infection of hepatitis B virus may cause an immunodeficient state against HBsAg. Therefore, hepatitis B virus containing cells cannot be removed by the usual mechanisms of cellular and humoral immunity. In fact, HBsAg-containing cells are seen most numerously in non-cancerous portion of the liver with hepatoma. Persistent presence of hepatitis B virus in hepatocytes may lead to malignant change of the hepatocytes.

If viral DNA integrate into genome of the liver cell during usual replication of HBV in acute hepatitis, this viral DNA probably does not have oncogene. Viral DNA is too small to contain oncogene.

However, persistent infection of hepatitis B virus and repeated integration and disintegration of viral DNA could cause disarrangement of host DNA, and produce malignant change. Well-differentiated hepatoma might have similar DNA composition as normal hepatocyte and still reproducible in tumor cells.

Table 1

Summary of data on chimpanzees inoculated with HBAg-containing sera.

No.	Inocula	Dilution	Week indicated finding in sera					Hepatic histopath.
			HBsAg	eAg	anti HBs	anti HBc	S-GPT (Max)	
1	adr eAg (+) DNA polymerase (+)	Undiluted	1	-	7-	20-	Normal	–
10		10^{-1}	4-7	-	11-	13-	7–9(378)	+
5	adr eAg (+) DNA polymerase (+) pooled	10^{-4}	8-25	11-17	-	14-	12–24(690)	+
6		10^{-8}	13-29	16-25	-	19-	18–26(460)	+
2	adr anti-e (+) DNA polymerase (–)	Undiluted	19-20	-	21-	21-	Normal	–
9	adr anti-e (+) DNA polymerase (–) pooled	10^{-2}	-	-	-	-	-	–
14		10^{-5}	-	-	-	-	-	–